

Green Synthesis, Characterization, and Biological Evaluation of Silver Nanoparticles Using *Bassia Scoparia* L. Leaves Extract

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Abstract

Silver nanoparticles (AgNPs) are known for their unique properties and applications in fields such as medicine and environmental remediation. Recent environmental concerns and the need for sustainable technologies have spurred interest in eco-friendly synthesis methods, particularly green synthesis using plant extracts, which is a cost-effective and biocompatible alternative to traditional physical and chemical methods. Silver nanoparticles (AgNPs) were synthesized using an aqueous leaf extract of *Bassia scoparia* L. as a reducing and stabilizing agent, demonstrating a simple and sustainable method for nanoparticle production. Comprehensive characterization was performed using various techniques: Scanning Electron Microscopy (SEM) assessed surface morphology and size distribution, X-ray Diffraction (XRD) confirmed crystallinity, Fourier Transform Infrared Spectroscopy (FTIR) identified functional groups involved in synthesis, Ultraviolet-Visible (UV-Vis) Spectroscopy monitored surface plasmon resonance, and Zeta Potential analysis examined surface charge and colloidal stability of the AgNPs. The results confirm the successful biosynthesis of stable, crystalline silver nanoparticles, demonstrating the potential of *Bassia scoparia* L. as an effective plant source for green nanotechnology applications.

Keywords: *Bassia Scoparia*, AgNPs, XRD, SEM, FTIR, Unique Properties, Spectroscopy

1. Introduction

Chemistry, physics, pharmaceutical sciences, materials science, medicine, and agriculture are all included in the multidisciplinary discipline of nanotechnology. Precision farming, which maximizes resource utilization by examining crop production variability, is one way that its implementation has significantly improved agriculture. For precision agriculture to advance and environmental sustainability to be promoted, nanotechnology must be incorporated into farming operations [1]. Nanoparticles, due to their small size, high surface area, and unique optical properties,

are utilized in agriculture for plant protection and nutrient delivery [2]. Nanoparticles, ranging in size from 1 to 100 nm, are composed of either dissolved, encapsulated, or integrated active compounds. They are fabricated into structures like Nano spheres and Nano capsules; the latter enclose substances within a non-toxic polymer shell, while the former allows for uniform distribution. Biodegradable polymer-based nanoparticles are particularly valuable for delivering proteins, genes, and peptides, especially those coated with hydrophilic polymers like polyethylene glycol (PEG). These serve as long-circulating particles. Nanoparticles

are being investigated for targeted drug delivery and gene therapy due to their capability to target specific organs, sustain prolonged circulation, and assist with DNA transport [3].

Nanoscale metal oxide particles, particularly titanium dioxide (TiO₂), are widely used in products such as toothpaste, cosmetics, sunscreens, and textiles. Silver (Ag) nano-powders are particularly noted for their strong antibacterial properties [4]. Metal oxides are preferred to organic antibacterial agents due to their greater safety and stability [5]. A Various physical and chemical methods are used to synthesize metal nanoparticles, but these are often costly, complex, and harmful to the environment. Consequently, there is a growing interest in sustainable and eco-friendly alternatives. Recent studies show that biological systems, particularly plants and algae, can effectively reduce inorganic metal ions to create metal nanoparticles. Among these methods, plant-based synthesis stands out for its higher production yields, safety, quicker synthesis, and lower cultivation costs [6].

Recent research highlights the significant role of microorganisms and biological entities in the synthesis of metal nanoparticles, moving away from traditional physical and chemical methods. The adoption of biological systems is favored for their simplicity, effectiveness, and alignment with green chemistry principles, as they avoid toxic and hazardous reagents [7]. Microorganisms with specific morphologies can create inorganic compounds at the nanoscale and demonstrate resistance to heavy metals through mechanisms like chemical detoxification and active ion transport via membrane proteins, such as ATPases, chemiosmotic cation channels, or proton antiporters. The solubility of these compounds is a key factor in microbial resistance [8,9]. Microbial systems effectively detoxify metal ions by converting soluble toxic ions into insoluble, non-toxic metallic nanoclusters through intracellular bioaccumulation and extracellular mechanisms like biomineralization and biosorption. Extracellular metal nanoparticle synthesis has significant industrial applications, with a focus on achieving monodispersity, as polydispersity is a major challenge. Fungi are highlighted as advantageous microbial candidates for intracellular nanoparticle synthesis due to their consistent sizing and reduced polydispersity.

Their networks withstand flow stress, agitation, and other bioreactor conditions better than both plant-based systems and bacterial cells. Fungi are also slow-growing, easier to control, and simpler to cultivate. Additionally, they secrete more reductive proteins that aid extracellular synthesis, facilitating easier downstream processing. Because the nanoparticles precipitate outside the cell, they are free from intracellular contaminants, making them immediately suitable for diverse practical applications [10]. AgNPs, typically 1–100 nm in size, offer a higher surface area and enhanced reactivity compared to bulk silver. Their unique electrical, optical, and catalytic properties have driven research into their use for drug delivery, diagnostics, and imaging [11,12]. Most notably, AgNPs exhibit strong antibacterial activity even against multidrug-resistant pathogens [13,14]. This enhanced

efficacy has facilitated the integration of silver nanoparticles (AgNP) in various healthcare and hygiene products, including surgical tools, cosmetics, dental materials, catheters, and dressings [15-18]. Their multiple mechanisms of action make them effective antibiotic alternatives by targeting various microbial structures simultaneously [19].

Silver nanoparticles (AgNPs) offer a promising alternative in the face of rising antibiotic resistance and the slow, costly development of new antibiotics, as they are effective in preventing and treating infections, decontaminating medical equipment, and addressing resistant microorganisms [20,21]. The increasing production of silver nanoparticles (AgNPs), now exceeding 500 tons annually, indicates a growing industrial demand. This surge has prompted greater attention to understanding their biological activity, mechanisms of action, and safety for both human and environmental health [22]. *Bassia scoparia* L. Voss, previously known as *Kochia*, is found in temperate and subtropical regions worldwide, though its exact Eurasian origin is unclear. It can be recognized by its annual life cycle, bushy form, flat leaves with petiole-like bases, leafy inflorescences, and distinctive fruiting perianth that may have tubercles or short wings. The species demonstrates considerable morphological variation, particularly in leaf shape, hair tufts at bract bases, and perianth structure [23,24].

2. Methods and Materials

2.1. Collection of Plant Materials

The study involved the collection of a plant species from the Tazagram region in District Dir Lower, Khyber Pakhtunkhwa, Pakistan. The specimen underwent taxonomic identification and authentication at the Department of Botany, GDC Gulabad, where expert botanists verified its identity using morphological and taxonomic keys. A voucher specimen was prepared and deposited in the herbarium to facilitate future comparative studies and maintain scientific integrity [25].

2.2. Preparation of Plant Extract

After identification and authentication, *Bassia scoparia* L. leaves were washed with distilled water to remove contaminants. They were shaded and air-dried at room temperature for several days to preserve phytochemical constituents, then ground into a fine powder using a mechanical grinder the extraction process involved transferring powdered leaves into a sealed glass container, followed by the addition of distilled water in a specific ratio for aqueous extraction. The mixture was incubated in an orbital shaker at a controlled temperature for 24 to 48 hours to extract bioactive compounds. After incubation, the mixture was filtered through Whatman No. 1 filter paper to remove solid residues, and the clear filtrate was stored at 4°C for silver nanoparticles synthesis [26].

2.3. Biosynthesis of AgNPs Mediated by *Bassia Scoparia* Leaves Extract

The green synthesis of silver nanoparticles (AgNPs) was conducted by mixing *Bassia scoparia* L. leaf extract with an aqueous

silver nitrate (AgNO₃) solution. The leaf extract, containing phytochemicals, was added dropwise to the AgNO₃ solution while continuously stirring at room temperature. This process allowed for the bioreduction of silver ions (Ag⁺) to metallic silver (Ag⁰) over several hours. A visual indicator of nanoparticle formation was the gradual color change of the solution from pale yellow to dark brown, serving as preliminary confirmation. This was further validated through spectroscopic and microscopic characterization techniques [26].

2.4. Characterization of the Synthesized AgNPs

2.4.1. UV-Vis Spectroscopy

Optical measurements were conducted with a Lambda 35 UV-Vis spectrophotometer, using distilled water as the reference solvent in quartz cuvettes. A 1 mL sample of the reaction mixture was diluted in 9 mL of water and sonicated for 15 minutes for UV measurements. Additionally, 2 mL of pure Ag NP stock solution was diluted with 8 mL of water for analysis, and a stock solution was prepared by dissolving 5 mg of silver nanoparticles in 5 mL of water and sonicating for one hour.

2.4.2. SEM Analysis

The surface morphology of the synthesized nanoparticles was analyzed using a Hitachi S-4500 scanning electron microscope. The sample underwent centrifugation at 14,000 rpm for 10 minutes, with the nanoparticle pellet resuspended in deionized water and recentrifuged three times, followed by an acetone wash. To achieve a uniform and stable suspension, the purified silver nanoparticles were sonicated for 30 minutes before drying the sample. A small dried sample was placed on a SEM grid to create a thin film, coated with gold via sputter coater, dried under a mercury lamp for 10 minutes, and SEM images were captured at various magnifications.

2.4.3. X-Ray Diffraction Spectroscopy Analysis

Cu K α radiation ($\lambda = 1.5418 \text{ \AA}$) was used to create XRD patterns on an Ultima IV X-ray powder diffractometer (Rigaku, Tokyo,

Japan).

2.4.4. Fourier-Transform Infrared Spectroscopy (FTIR)

A PerkinElmer 1000 FT-IR spectrometer was employed to acquire FT-IR spectra. The Ag NPs were purified with distilled water to remove free biomass and unbound extract. The final product was centrifuged for 30 minutes at 9000 rpm and dried. For measurement, the cleaned Ag NPs were mixed with KBr powder and pressed into a pellet, using a reference blank KBr pellet to adjust the background.

2.4.5. Antibacterial Assay

The antibacterial activity of the silver nanoparticle-synthesized extract was evaluated using the Microplate Alamar Blue Assay against various antibacterial strains. Sterilized Petri dishes with nutrient agar were prepared, and a standardized 24-hour bacterial culture was spread on the agar. Wells were created, and 50 μL of the extracts was introduced into each. After incubation at 37 °C for 24 hours, antibacterial activity was assessed by measuring the inhibition zones around the wells.

2.5. Antifungal Assay

The agar tube dilution method was utilized to assess the antifungal activity of silver nanoparticles (AgNPs) derived from the leaf extracts of *Bassia scoparia* L. Six fungal strains were tested: *Trichophyton rubrum*, *Candida albicans*, *Aspergillus niger*, *Microsporum canis*, *Fusarium lini*, and *Candida glabrata*. A mixture of 25 ml distilled water and 25 mg of the AgNPs pellet was prepared to achieve a final concentration of 1000 ppm. Fungal growth medium was prepared and sterilized by autoclaving at 121 °C for 20 minutes. Under aseptic conditions in a laminar flow hood, 4 mL of autoclaved Sabouraud Dextrose Agar was dispensed into test tubes, which were tilted to form slants. Fungal cultures were inoculated on the slants, with miconazole as a positive control and dimethyl sulfoxide (DMSO) as a negative control. The tubes were incubated at 27 °C for 7 days to evaluate antifungal activity using a percentage inhibition formula.

$$\text{Percentage inhibition (\%)} = \frac{\text{Linear growth in negative control} - \text{Linear growth in sample}}{\text{Linear growth in negative control}} \times 100$$

A negative control reading served as a reference for evaluating fungal growth, with percentage inhibition calculated using a specified formula [27].

3. Result and Discussion

3.1. Ultraviolet Visible Spectroscopy (UV-Vis spectroscopy) Analysis

The UV-Vis absorption spectra of nanoparticles synthesized from *Bassia scoparia* leaf extract reveal a strong peak at 405 nm and a secondary shoulder at 414 nm. The prominent 405 nm peak indicates the formation of small, stable spherical nanoparticles, while the 414 nm shoulder suggests variations in size or morphology.

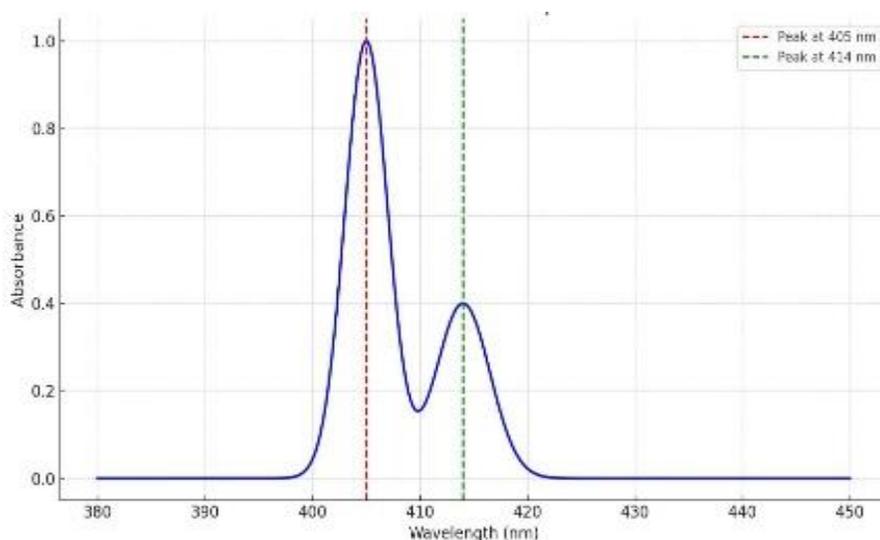


Figure 1: UV-Vis Absorption Spectra of Green Synthesized Nanoparticles from *Bassia Scoparia* L. Leaf Extract

3.2. SEM Analysis

The SEM micrograph of synthesized silver nanoparticles shows predominantly spherical morphologies with an average diameter of approximately 30 nm, although some irregularities exist. Larger particles observed are likely due to the aggregation of smaller nanoparticles, highlighting the morphology of biologically synthesized silver nanoparticles. SEM was utilized to analyze the size, shape, and surface morphology of biologically synthesized

silver nanoparticles (AgNPs). This electron microscopy technique provides high-resolution images, with magnification from 20X to 30,000X and a resolution of 50 to 100 nm. Earlier studies reported that silver nanoparticles made with *Aloe vera* extract ranged from 9 to 18 nm in size and typically exhibited hexagonal geometry, while zinc oxide nanoparticles from *Glycosmis pentaphylla* leaf extract were mostly spherical [28].

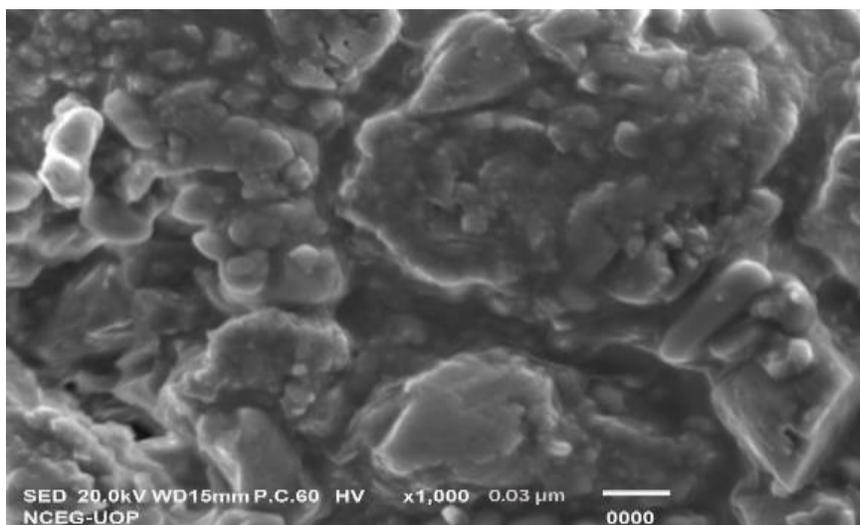


Figure 2: SEM Micrograph of Green Synthesized Nanoparticles from *Bassia Scoparia* Leaf Extract

3.3. XRD Analysis

X-ray diffraction (XRD) analysis identified the crystalline phase of green-synthesized silver nanoparticles (AgNPs), revealing six distinct peaks corresponding to various lattice planes at specific

2θ values. These peaks confirmed the face-centered cubic (FCC) structure of silver, as per JCPDS standards. Unassigned peaks were also noted, likely due to bio-organic compound crystallization from *Phlomis* extract on the nanoparticle surface.

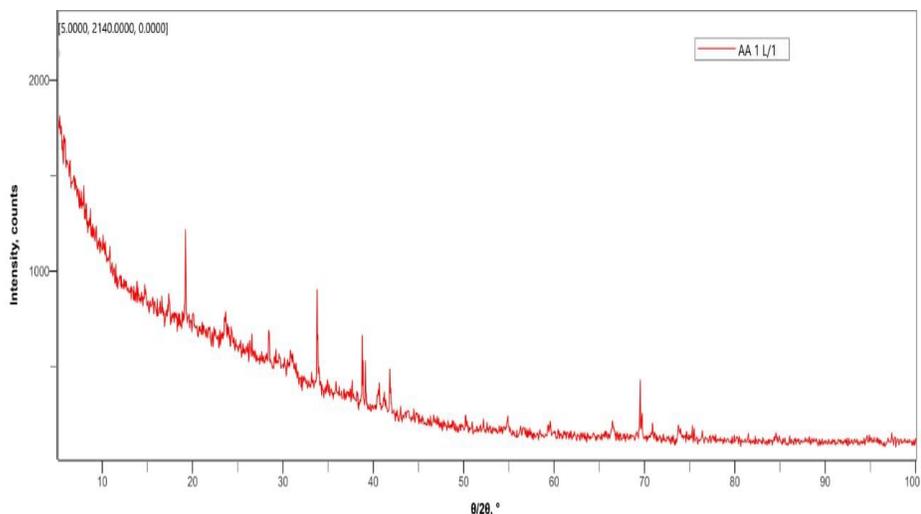


Figure 3: XRD Pattern of Green Synthesized Nanoparticles from *Bassia Scoparia* Leaf Extract

Using X-ray diffraction, the structure, purity, and phase identity of green-produced AgNPs were ascertained [29]. A previous work using *G. ofcinalis* plant extract where XRD peaks in degrees 2θ appear at 38.0946° , 41.4385° , 64.494° , and 77.349° this can be attributed to the planes (111), (200), (220), (311) and (222) sets of lattice planes of crystal [30]. The XRD pattern confirmed the crystalline nature of the synthesized silver nanoparticles, with sharp diffraction peaks indicating that the particles are within the nanometer size range, aligning with standard reference data from the JCPDS [31].

3.4. FTIR Analysis

The low-frequency region between 894 and 484 cm^{-1} is associated with metal–oxygen linkages, confirming the interaction of silver with plant metabolites. These findings suggest that biomolecules such as polyphenols, proteins, and amines from *Bassia scoparia* leaf extract function both as reducing agents and as stabilizers during the nanoparticle synthesis process. The low-frequency region between 894 and 484 cm^{-1} indicates metal–oxygen linkages, confirming the interaction of silver with plant metabolites. Biomolecules from *Bassia scoparia* leaf extract, including polyphenols, proteins, and amines, act as reducing agents and stabilizers in nanoparticle synthesis.

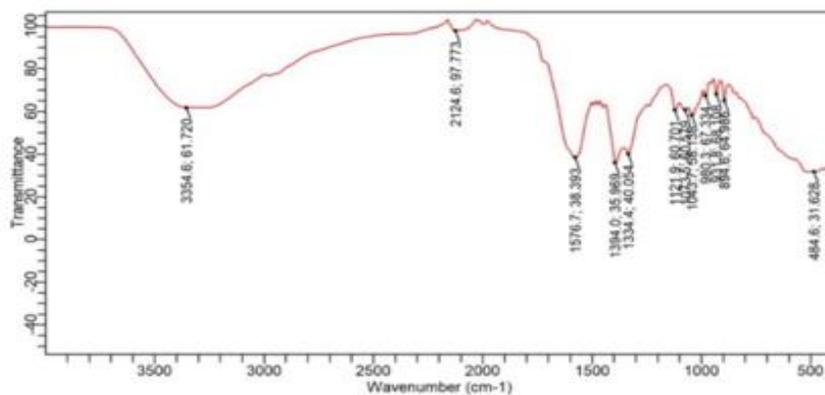


Figure 4: FTIR Spectra of Green Synthesized Silver Nanoparticles from *Bassia Scoparia* Leaf Extract

3.5. Antibacterial Assay

We used the Microplate Alamar Blue Assay to assess the antibacterial properties of silver nanoparticles synthesized with *Bassia scoparia* L. leaf extract against five bacterial strains: *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Salmonella typhi*. The results indicated that the AgNPs had the highest inhibition rates of 89% against *E. coli* and 69% against *P. aeruginosa*, while the standard drug showed 91% and 78% inhibition, respectively. No antibacterial activity was detected against *B. subtilis*, *S. aureus*, or *S. typhi*. These findings suggest that *Bassia scoparia* L. mediated

AgNPs are selectively effective against specific gram-negative bacteria, notably *E. coli* and *P. aeruginosa*, but demonstrate limited or no activity against the tested gram-positive strains. AgNPs synthesized from *Acer oblongifolium* extract demonstrated strong antibacterial properties, evidenced by inhibition zones of 13–26 mm in disc diffusion assays against multiple strains [32]. Another study demonstrated that AgNPs from *Cinnamomum tamala* showed significant inhibitory effects against *P. aeruginosa*. This supports our results, highlighting that smaller AgNPs, capped with plant phytochemicals, have enhanced antibacterial efficacy, especially against gram-negative bacteria.

| Name of Bacteria | % Inhibition of Compound | % Inhibition of Drug |
|-------------------------------|--------------------------|----------------------|
| <i>Escherichia coli</i> | 89 | 91 |
| <i>Bacillus subtilis</i> | No activity | 89 |
| <i>Staphylococcus aureus</i> | No activity | 83 |
| <i>Pseudomonas aeruginosa</i> | 69 | 78 |
| <i>Salmonella typhi</i> | No activity | 84.3 |

Table 1: Antibacterial Activity of Green Synthesized Nanoparticles from *Bassia Scoparia* Leaf Extract Compared with Standard Drug

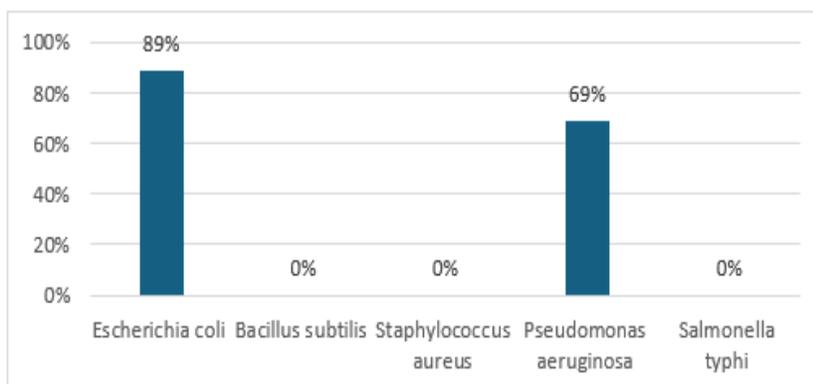


Figure 5: Comparative Antibacterial Activity of Green Synthesized Nanoparticles from *Bassia Scoparia* L. Leaf Extract and Standard Drug Against Selected Bacterial Strains

3.6. Antifungal Assay

An antifungal assay evaluated the effectiveness of biologically synthesized silver nanoparticles (AgNPs) using *Bassia scoparia* L. leaf extract against seven fungal pathogens: *Trichophyton rubrum*, *Candida albicans*, *Aspergillus niger*, *Microsporum canis*, *Fusarium lini*, *Candida glabrata*, and *Aspergillus fumigatus*. The inhibitory effect of AgNPs was measured by comparing the linear growth of fungal colonies with untreated controls. The results of this experiment are summarized in Table 2. The synthesized silver nanoparticles demonstrated significant antifungal activity, particularly against *Aspergillus fumigatus* (92% growth inhibition), *Candida glabrata* (89%), and *Aspergillus niger* (83%). Notable inhibition was also observed for *Fusarium lini* (75%), while moderate effects were noted against *Trichophyton rubrum*

(62%), *Microsporum canis* (55%), and *Candida albicans* (50%). The study highlights that silver nanoparticles synthesized from *Bassia scoparia* L. leaf extract exhibit significant antifungal activity, particularly against *Aspergillus fumigatus*, *Candida glabrata*, and *Aspergillus niger*. Miconazole and amphotericin B served as positive controls, while untreated samples were negative controls, indicating the potential of green-synthesized AgNPs for biomedical and agricultural applications. The study explores the synthesis of silver nanoparticles using *Bassia scoparia* L. leaf extract for antifungal applications. It references similar research with *Azadirachta indica* (neem), which showed significant antifungal activity against strains like *Aspergillus niger* and *Candida albicans*, demonstrating that plant-derived silver nanoparticles can effectively inhibit pathogenic fungi, with results

comparable to standard antifungal drugs [33].

| Name of Fungus | Linear Growth | (mm) | Inhibition% | Standard Drug |
|------------------------------|---------------|--------------|-------------|----------------|
| | Sample (mm) | Control (mm) | | |
| <i>Trichophyton rubrum</i> | 38 | 100 | 62% | Miconazole |
| <i>Candida albicans</i> | 50 | 100 | 50% | Miconazole |
| <i>Aspergillus niger</i> | 17 | 100 | 83% | Amphotericin-B |
| <i>Microsporium canis</i> | 45 | 100 | 55% | Miconazole |
| <i>Fusarium lini</i> | 25 | 100 | 75% | Miconazole |
| <i>Candida glabrata</i> | 11 | 100 | 89% | Miconazole |
| <i>Aspergillus fumigatus</i> | 8 | 100 | 92% | Miconazole |

Table 2: Antifungal Activity of Green-Synthesized Nanoparticles from Bassia Scoparia Leaf Extract Compared with Standard Drug

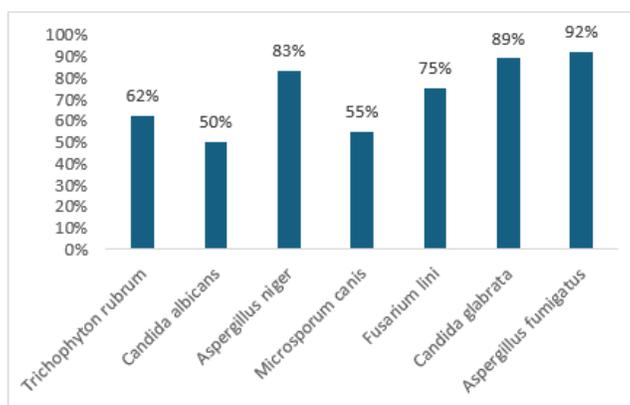


Figure 6: Comparative Antifungal Activity of Green Synthesized Nanoparticles from Bassia Scoparia L. Leaf Extract and Standard Drug Against Selected Bacterial Strains [34-37].

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